(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 February 2002 (07.02.2002)

PCT

(10) International Publication Number
WO 02/09668 A2

(51) International Patent Classification[7]: A61K 9/00

(21) International Application Number: PCT/US01/21105

(22) International Filing Date: 20 July 2001 (20.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/222,476 2 August 2000 (02.08.2000) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/09668 A2

(54) Title: PULMONARY ADMINISTRATION OF FLINT

(57) Abstract: The claimed invention relates to methods and compositions for administering FLINT by inhalation, and a methods for treating a disease by administering FLINT by inhalation.

PULMONARY ADMINISTRATION OF FLINT

Field of the Invention

This invention relates to the pulmonary delivery of FLINT for systemic absorption through the lungs to significantly reduce or eliminate the need for administering FLINT by injection or other routes of administration.

Background of the Invention

A number of proteins homologous to tumor necrosis factor receptor proteins ("TNFR proteins") have been isolated in recent years, having many potent biological effects. Aberrant activity of these proteins has been implicated in a number of disease states.

A recently discovered TNFR homologue, referred to herein as "FAS Ligand Inhibitory Protein," or "FLINT", binds Fas Ligand (FasL) to prevent the interaction of FasL with Fas (See PCT Application No. WO 99/50413).

Increased activation of the Fas-FasL signal transduction pathway is thought to underlie a number of pathological conditions, including runaway apoptosis (Kondo *et al.*, Nature Medicine 3(4):409-413 (1997); Galle *et al.*, *J. Exp. Med.* 182:1223-1230 (1995)), and inflammatory disease resulting from neutrophil activation (Miwa *et al.*, *Nature Medicine 4*:1287 (1998)).

Runaway apoptosis signifies a level of apoptosis greater than normal, or apoptosis occurring at an inappropriate time. Pathological conditions caused by runaway apoptosis include, for example, organ failure in the liver, kidneys and pancreas. Inflammatory diseases associated with excessive neutrophil activation include sepsis, ARDS, SIRS and MODS.

FLINT inhibits not only the binding of Fas to FasL, but also the binding of another ligand, LIGHT, to LTβR, and/or TR2/HVEM receptors. This property can be used to treat or prevent diseases or conditions that may be associated with these binding interactions.

Like many other proteins, FLINT is thought to rapidly degrade in the gastrointestinal tract, and therefore FLINT is not efficiently absorbed from the GI tract into the blood stream. Therefore, exogenous administration of FLINT for therapeutic purposes depends, in most instances, upon injection treatments. Alternatively, some proteins may be efficiently administered by rectal, transdermal, and nasal routes. Thus far, however, these routes of administration have not resulted in generally effective protein absorption.

Over the years, certain drugs have been sold in compositions suitable for forming a drug dispersion for oral inhalation (pulmonary delivery) to treat various conditions in humans. Such pulmonary drug delivery compositions are designed to be delivered by inhalation of a drug dispersion by the patient so that the active drug within the dispersion can reach the lung. It has been found that certain drugs delivered to the lung are readily absorbed through the alveolar region directly into blood circulation.

Pulmonary delivery is particularly promising for the delivery of proteins and polypeptides which are difficult to deliver by other routes of administration. Such pulmonary delivery can be effective both for systemic delivery and for localized delivery to treat diseases of the lungs.

Pulmonary drug delivery can be achieved by different approaches, including liquid nebulizers, aerosol-based metered dose inhalers (MDI's), and dry powder dispersion devices. Dry powder dispersion devices, which do not rely on

CFC aerosol technology, are promising for delivering drugs that may be readily formulated as dry powders, particularly proteins and polypeptides. Many otherwise labile proteins and polypeptides may be stably stored as lyophilized or spray-dried powders by themselves or in combination with suitable powder carriers.

The ability to deliver proteins and polypeptides as dry powders, however, is problematic in certain respects. The dosage of many protein and polypeptide drugs is often critical so it is necessary that any dry powder delivery system be able to accurately, precisely, and reliably deliver the intended amount of drug. Moreover, many proteins and polypeptides are quite expensive, typically being many times more costly than conventional drugs on a per-dose basis. Thus, the ability to efficiently deliver the dry powders with a minimal loss of drug is critical.

It is now known that effective absorption of proteins can occur from the lung. For example, administration of insulin as an inhalation aerosol to the lung was first reported by Gaensslen in 1925. Recently, pulmonary administration of erythropoietin has been described (United States Patent No. 5,354,934).

The efficiency of pulmonary administration depends on numerous factors. To a large extent, pulmonary absorption is dependent on the physical characteristics of the particular therapeutic protein to be delivered. Moreover, efficacy is at least partly dependent on the ability to deliver the protein to the deep lung alveolar epithelium. Proteins that are deposited in the upper airway epithelium are not absorbed to a significant extent. This is due to the overlying mucus which is approximately 30 μm - 40 μm thick, which acts as a barrier to absorption. In addition,

proteins deposited on this epithelium are cleared by mucociliary transport up the airways and then eliminated via the gastrointestinal tract.

FLINT, as a therapeutic protein, is useful in treating and/or preventing diseases that may relate to enhanced apoptosis. Therapeutic administration of FLINT can be accomplished by any number of suitable methods, including, for example, intravenous and subcutaneous administration. However, such treatments can be painful to a patient, especially to one who must receive multiple dosings for effective treatment. Thus, there is a need for a route of therapeutic administration of FLINT that minimizes the pain and discomfort of injection methods.

Summary of the Invention

The present invention solves this problem by administering FLINT comprising administering by pulmonary means an effective amount of FLINT to a patient in need thereof.

The present invention also relates to a method for treating a disease related to enhanced apoptosis comprising administering by pulmonary means an effective dose of FLINT to a patient in need thereof. Preferably, FLINT is delivered by inhalation, to the lower airway of the patient.

A further object of this invention is to provide a powdered pharmaceutical composition containing FLINT suitable for pulmonary delivery.

The FLINT compounds of the invention can be delivered to the lung in a carrier, as a solution or suspension, or as a dry powder, using any of a variety of devices suitable for pulmonary administration by inhalation. Preferably, a FLINT compound is delivered at a particle size effective for

reaching the lower airways of the lung. A preferred particle size is below 10 microns. An even more preferred particle size is between 1 and 5 microns.

Detailed Description of the Invention

The term "powder" or "powdered" refers to a composition that consists of finely dispersed solid particles that are relatively free flowing and capable of being dispersed in an inhalation device and subsequently inhaled by a subject so that the particles reach the lungs to permit penetration into the alveoli. Thus, the powder is administrable by inhalation therapy and is said to be "respirable" and suitable for pulmonary delivery. In general, the average particle size is less than about 10 microns (μm) in diameter and the particle shapes may be irregular, uniform or mixed. Preferably, the average particle size is less than about 7.5 μm and more preferably less than about 5.0 μm. Usually the particle size distribution is between about 0.1 μm and about 5 μm in diameter, particularly between about 2 μm to about 5 μm.

The term "dry" means that the powder composition has a moisture content such that the particles are readily dispersible in an inhalation device to form an aerosol. This moisture content is generally below about 10% by weight (%w) water, usually below about 5%w and preferably less than about 3%w.

The term "dispersibility" means the degree to which a powder composition can be dispersed (i.e. suspended) in a current of air so that the dispersed particles can be respired or inhaled into the lungs of a subject. For example, a powder composition that is only 10% dispersible means that only 10% of the mass of finely-divided particles

making up the composition can be suspended for inhalation into the lungs; 50% dispersibility means that 50% of the mass can be suspended. A standard measurement of dispersibility is described hereinafter.

The term "therapeutically effective amount" is the amount of FLINT present in the powder composition that is needed to provide the desired level to a subject to be treated to give the anticipated physiological response.

The term "physiologically effective amount" is that amount delivered to a subject to give the desired palliative or curative effect. This amount is specific for each active agent and its ultimately approved dosage level.

The term "pharmaceutically acceptable" refers to an excipient, whether a carrier or a protein used to improve dispersibility, that can be taken into the lungs with no significant adverse toxicological effects on the lungs.

The term "FLINT" as used herein refers to native FLINT (SEQ ID NO:3), mature FLINT lacking the leader sequence (SEQ ID NO:1) and/or to FLINT analogs. The term "protease-resistant analog" or "protease-resistant FLINT analog" means a variant FLINT sequence, or fragment thereof, resistant to proteolysis between positions 218 and 219 of SEQ ID NO:1 (positions 247 and 248 of SEQ ID NO:3). Protease resistant analogs may have one, two, three, or more amino acid substitutions, deletions, additions, or other changes to the sequence of SEQ ID NO:1 or SEQ ID NO:3. Preferably, there is a single substitution in said sequence.

The term "FLINT analog" or "analog" refers to a variant FLINT, or fragment thereof, preferably having a biological activity substantially the same as FLINT, for example, the ability to inhibit apoptosis in vitro and/or in vivo. A FLINT analog comprises one or more substitutions, additions,

deletions in the amino acid sequence embodied in SEQ ID NO:1 or 3.

The term "protease-resistant" refers to a FLINT analog that, when compared with FLINT, or FLINT fragment, exhibits resistance to proteolysis between residues 218 and 219 of SEQ ID NO:1. Protease resistant analogs differ from FLINT by one or more amino acid substitutions, deletions, inversions, additions, and/or changes in glycosylation sites, or patterns, as compared with native FLINT, or mature FLINT, or FLINT fragment. Preferably these changes occur in the region from about position 214 through position 222 of SEQ ID NO:1.

The term "protease-resistant" contemplates degrees of resistance to proteolysis at position 218 from complete resistance to partial resistance. Thus, a "resistant analog" may show a degree of resistance to proteolysis at position 218, as measured by half-life, that is at least about 25% greater than native FLINT when treated or exposed to a suitable protease. Preferably, a resistant FLINT analog possesses a protease resistance half-life that is at least about 2-fold greater than native FLINT.

As used herein "half-life" refers to the time required for approximately half of FLINT or a FLINT analog molecule in a sample to be proteolytically cleaved between positions 218 and 219 of SEQ ID NO:1, *in vitro* and/or *in vivo*, as determined by any suitable means.

Exemplary protease-resistant FLINT analogs include the following substitutions in SEQ ID NO:1:

a. Arg at position 218 is replaced by Gln;

b. Arg at position 218 is replaced by Glu;

c. Thr at position 216 is replaced by Pro;

d. Arg at position 218 is replaced by Ala;

e. Arg at position 218 is replaced by Gly;

f. Arg at position 218 is replaced by Ser;

g. Arg at position 218 is replaced by Val

h. Arg at position 218 is replaced by Tyr;

i. Pro at position 217 is replaced by Tyr

j. Thr at position 216 is replaced by Pro, and Arg at position 218 is replaced by Gln.

Additional protease-resistant FLINT analogs are disclosed in PCT Patent Application Serial No. PCT/US 00/06418.

The term "preservative" refers to a compound added to a pharmaceutical formulation to act as an anti-microbial agent. A parenteral formulation must meet guidelines for preservative effectiveness to be a commercially viable multi-use product. Among preservatives known in the art as being effective and acceptable in parenteral formulations are benzalkonium chloride, benzethonium, chlorohexidine, phenol, m-cresol, benzyl alcohol, methylparaben, chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. *See, e.g.*, Wallhäusser, K.-H., *Develop. Biol. Standard,* 24: 9-28 (Basel, S. Krager, 1974).

The term "buffer" or "pharmaceutically acceptable buffer" refers to a compound that is safe for use in FLINT formulations and has the effect of controlling the pH of the formulation at the pH desired for the formulation. Pharmaceutically acceptable buffers for controlling pH at a moderately acid pH to a moderately basic pH include, for example, such compounds as phosphate, acetate, citrate, TRIS, arginine, or histidine.

The term "isotonicity agent" refers to a compound that is tolerated physiologically and imparts a suitable tonicity to a formulation to prevent the net flow of water across the cell membrane. Compounds such as glycerin are commonly used

for such purposes at known concentrations. Other acceptable isotonicity agents include physiological solutions of salts, *e.g.*, NaCl, dextrose, mannitol, and lactose. Glycerol at a concentration of 12 to 25 mg/mL is preferred as an isotonicity agent.

Pulmonary Administration of FLINT

FLINT is administered by inhalation in a dose effective manner to increase circulating FLINT protein levels. Administration of FLINT can be effective for treating a variety of disorders such as, for example, inflammatory diseases associated with neutrophil activation including sepsis, acute respiratory distress syndrome, acute lung injury, systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction (MODS).

Other diseases for which FLINT could be therapeutically effective include rheumatoid arthritis (Elliott *et al.*, *Lancet* 344:1105-10 (1994)), fibroproliferative lung disease, fibrotic lung disease, HIV (Dockrell *et al.*, *J. Clin. Invest.* 101:2394-2405 (1998)), ischemia (Sakurai et al. 1998 Brain Res 797:23-28), brain trauma/injury (Ertel et al. 1997 J Neuroimmunol 80:93-6), chronic renal failure (Schelling et al. 1998 Lab Invest 78:813-824), Graft-vs-Host Disease (GVHD) (Hattori et al. 1998 Blood 11:4051-4055), Cutaneous inflammation (Orteu at al. 1998 J Immunol 161:1619-1629), Vascular leak syndrome (Rafi et al. 1998 J Immunol 161:3077-3086), Helicobacter pylori infection (Rudi et al. 1998 J Clin Invest 102:1506-1514), Goiter (Tamura et al. 1998 Endocrinology 139:3646-3653), Atherosclerosis (Sata and Walsh, 1998 J Clin Invest 102:1682-1689), IDDM (Itoh et al. 1997 J Exp Med 186:613-618), Osteoporosis (Jilka et al. 1998 J Bone Min Res 13:793-802), Crohn's

Disease (van Dullemen et al. 1995 Gastroenterology 109:129-35), organ preservation and transplant (graft) rejection (Lau et al. 1996 Science 273:109-112), Sepsis (Faist and Kim. 1998 New Horizons 6:S97-102), Pancreatitis (Neoptolemos et al. 1998 Gut 42:886-91), Cancer (melanoma, colon and esophageal) (Bennett et al. 1998 J Immunol 160:5669-5675), Autoimmune disease (IBD, psoriasis, Down's Syndrome (Seidi *et al., Neuroscience Lett. 260*:9 (1999) and multiple sclerosis (D'Souza et al. 1996 J Exp Med 184:2361-70); Alzheimer's Disease; End-stage renal disease (ESRD); mononulceosis; EBV; Herpes; antibody dependent sytotoxicity; hemolytic and hypercoagulation disorders such as vascular bleeds, DIC (disseminated intervascular coagulation), eclampsia, HELLP (preeclampsia complicated by thrombocytopenia, hemolysis and disturbed liver function), HITS (heparin induced thromobcytopenia), HUS (hemolytic uremic syndrome), and preeclampsia; hematopoeitic disorders such as aplastic anemia, thrombocytopenia (TTP) and myelodysplasia; and hemolytic fever caused, for example, by E.bola.

Achieving effective doses of FLINT requires administration of an inhaled dose of more than about 0.5 µg/kg to about 500 µg/kg FLINT protein; preferably about 3 µg/kg to about 250 µg/kg; and more preferably about 5 µg/kg to about 100 µg/kg; more preferably still from about 7 ug/kg to about 50 ug/kg; still more preferably from about 7 ug/kg to about 20 ug/kg; most preferably from about 7 ug/kg to about 14 ug/kg. A therapeutically effective amount of FLINT can be determined by a knowledgeable medical practitioner taking into account factors including the physical condition of the patient, the patient's pulmonary status, and the like.

According to the invention, FLINT is delivered by inhalation to achieve absorption of the protein. Administration by inhalation can result in pharmacokinetics comparable to subcutaneous administration. Different inhalation devices typically provide similar pharmacokinetics when similar particle sizes and similar levels of lung deposition are compared.

According to the invention, FLINT can be delivered by any of a variety of inhalation devices known in the art for administration of a therapeutic agent by inhalation. These devices include metered dose inhalers, nebulizers, dry powder generators, sprayers, and the like. Preferably, FLINT is delivered by a dry-powder inhaler, or sprayer. There are several desirable features of an inhalation device for administering FLINT. For example, delivery by the inhalation device is advantageously reliable, reproducible, and accurate. The inhalation device should deliver small particles, e.g. less than about 10 μm, preferably about 1-5 μm, for good respirability. Some specific examples of commercially available inhalation devices suitable for the practice of this invention are Turbohaler™ (Astra), Rotahaler® (Glaxo), Diskus® (Glaxo), Spiros™ inhaler (Dura), devices marketed by Inhale Therapeutics, AERx™ (Aradigm), the Ultravent® nebulizer (Mallinckrodt), the Acorn II® nebulizer (Marquest Medical Products), the Ventolin® metered dose inhaler (Glaxo), the Spinhaler® powder inhaler (Fisons), or the like.

As those skilled in the art will recognize, the formulation of FLINT protein, the quantity of the formulation delivered, and the duration of administration of a single dose depend on the type of inhalation device employed. For some aerosol delivery systems, such as

nebulizers, the frequency of administration and length of time for which the system is activated will depend mainly on the concentration of FLINT in the aerosol. For example, shorter periods of administration can be used at higher concentrations of protein in the nebulizer solution. Devices such as metered dose inhalers can produce higher aerosol concentrations, and can be operated for shorter periods to deliver the desired amount of protein. Devices such as powder inhalers deliver active agent until a given charge of agent is expelled from the device. In this type of inhaler, the amount of FLINT protein in a given quantity of the powder determines the dose delivered in a single administration.

The particle size of the FLINT protein in the formulation delivered by the inhalation device is important with respect to the ability of protein to make it into the lungs, and preferably into the lower airways or alveoli. Preferably, the FLINT is formulated so that at least about 10% of the protein delivered is deposited in the lung, preferably about 10% to about 20%, or more. It is known that the maximum efficiency of pulmonary deposition is obtained with particle sizes of about 2 μm to about 3 μm. When particle sizes are above about 5 μm, pulmonary deposition decreases substantially. Particle sizes below about 1 μm cause pulmonary deposition to decrease, and it becomes difficult to deliver particles with sufficient mass to be therapeutically effective. Thus, particles of FLINT protein delivered by inhalation have a particle size preferably less than about 10 μm, more preferably in the range of about 1 μm to about 5 μm, and most preferably in the range of about 2 μm to about 3 μm. The formulation of

FLINT protein is selected to yield the desired particle size in the chosen inhalation device.

Administration of FLINT by a Dry Powder Inhaler

Advantageously for administration as a dry powder, FLINT is prepared in a particulate form with a particle size of less than about 10 μm, preferably about 1 to about 5 μm, and most preferably about 2 μm to about 3 μm. The preferred particle size is effective for delivery to the alveoli of the patient's lung. Preferably, the dry powder is largely composed of particles produced so that a majority of the particles have a size in the desired range. Advantageously, at least about 50% of the dry powder is made of particles having a diameter less than about 10 μm. Such formulations can be achieved by spray drying, milling, or critical point condensation of a solution containing FLINT and other desired ingredients. Other methods also suitable for generating particles useful in the current invention are known in the art.

The particles are usually separated from a dry powder formulation in a container and then transported into the lung of a patient via a carrier air stream. Typically, in current dry powder inhalers, the force for breaking up the solid is provided solely by the patient's inhalation. One suitable dry powder inhaler is the Turbohaler™ manufactured by Astra (Södertalje, Sweden). In another type of inhaler, air flow generated by the patient's inhalation activates an impeller motor which deagglomerates the protein particles. The Dura Spiros™ inhaler is such a device.

Formulations of FLINT for administration from a dry powder inhaler typically include a finely divided dry powder containing FLINT protein, but the powder can also include a

bulking agent, carrier, excipient, another additive, or the like. Additives can be included in a dry powder formulation of protein, for example, to dilute the powder as required for delivery from the particular powder inhaler, to facilitate processing of the formulation, to provide advantageous powder properties to the formulation, to facilitate dispersion of the powder from the inhalation device, to stabilize the formulation (e.g., antioxidants or buffers), to provide taste to the formulation, or the like. Advantageously, the additive does not adversely affect the patient's airways. The FLINT protein can be mixed with an additive at a molecular level or the solid formulation can include particles of the FLINT protein mixed with or coated on particles of the additive. Typical additives include mono-, di-, and polysaccharides; sugar alcohols and other polyols, such as, for example, lactose, glucose, raffinose, melezitose, lactitol, maltitol, trehalose, sucrose, mannitol, starch, or combinations thereof; surfactants, such as sorbitols, diphosphatidyl choline, or lecithin; or the like. Typically an additive, such as a bulking agent, is present in an amount effective for a purpose described above, often at about 50% to about 90% by weight of the formulation. Additional agents known in the art for formulation of a protein can also be included in the formulation.

Administration of FLINT as a Spray

A spray including FLINT protein can be produced by forcing a suspension or solution of FLINT protein through a nozzle under pressure. The nozzle size and configuration, the applied pressure, and the liquid feed rate can be chosen to achieve the desired output and particle size. An

electrospray can be produced, for example, by an electric field in connection with a capillary or nozzle feed. Advantageously, particles of FLINT delivered by a sprayer have a particle size less than about 10 μm, preferably in the range of about 1 μm to about 5 μm, and most preferably about 2 μm to about 3 μm.

Formulations of FLINT protein suitable for use with a sprayer typically include protein in an aqueous solution at a concentration of about 1 mg to about 20 mg of FLINT protein per ml of solution. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the protein, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating FLINT include albumin, protamine, or the like. Typical carbohydrates useful in formulating FLINT proteins include sucrose, mannitol, lactose, trehalose, glucose, or the like. The FLINT protein formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of FLINT caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitol fatty acid esters. Amounts will generally range between 0.001 and 4% by weight of the formulation. Additional agents known in the art for formulation of a protein can also be included in the formulation.

Administration of FLINT by a Nebulizer

FLINT protein can be administered by a nebulizer, such as jet nebulizer or an ultrasonic nebulizer. Typically, in

a jet nebulizer, a compressed air source is used to create a high-velocity air jet through an orifice. As the gas expands beyond the nozzle, a low-pressure region is created, which draws a solution of FLINT protein through a capillary tube connected to a liquid reservoir. The liquid stream from the capillary tube is sheared into unstable filaments and droplets as it exits the tube, creating the aerosol. A range of configurations, flow rates, and baffle types can be employed to achieve the desired performance characteristics from a given jet nebulizer. In an ultrasonic nebulizer, high-frequency electrical energy is used to create vibrational, mechanical energy, typically employing a piezoelectric transducer. This energy is transmitted to the formulation of FLINT protein either directly or through a coupling fluid, creating an aerosol including the FLINT protein. Advantageously, particles of FLINT protein delivered by a nebulizer have a particle size less than about 10 μm, preferably in the range of about 1 μm to about 5 μm, and most preferably about 2 μm to about 3 μm.

Formulations of FLINT protein suitable for use with a nebulizer, either jet or ultrasonic, typically include FLINT protein in an aqueous solution at a concentration of about 1 mg to about 20 mg of FLINT protein per ml of solution. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the FLINT protein, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in such formulations include albumin, protamine, or the like. Typical carbohydrates useful in such formulations include sucrose, mannitol, lactose, trehalose, glucose, or the like. The

FLINT protein formulation can also include a surfactant, to reduce or prevent surface-induced aggregation of the FLINT protein caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbital fatty acid esters. Amounts will generally range between 0.001 and 4% by weight of the formulation. Additional agents known in the art for formulation of a protein can also be included in the formulation.

Administration of FLINT by a Metered Dose Inhaler

In a metered dose inhaler (MDI), a propellant, FLINT protein, and any excipients or other additives are contained in a canister as a mixture including a liquefied compressed gas. Actuation of the metering valve releases the mixture as an aerosol, preferably containing particles in the size range of less than about 10 μm, preferably about 1 μm to about 5 μm, and most preferably about 2 μm to about 3 μm. The desired aerosol particle size can be obtained by employing a formulation of FLINT protein produced by various methods known to those of skill in the art, including jet-milling, spray drying, critical point condensation, or the like. Preferred metered dose inhalers include those manufactured by 3M or Glaxo and employing a hydrofluorocarbon propellant.

Formulations of FLINT protein for use with a metered-dose inhaler device will generally include a finely divided powder containing FLINT protein as a suspension in a non aqueous medium, for example, suspended in a propellant with the aid of a surfactant. The propellant may be any conventional material employed for this purpose, such as

chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol and 1,1,1,2-tetrafluoroethane, HFA-134a (hydrofluroalkane-134a), HFA-227 (hydrofluroalkane-227), or the like. Preferably the propellant is a hydrofluorocarbon. The surfactant can be chosen to stabilize the FLINT protein as a suspension in the propellant, to protect the active agent against chemical degradation, and the like. Suitable surfactants include sorbitan trioleate, soya lecithin, oleic acid, or the like. In some cases solution aerosols are preferred using solvents such as ethanol. Additional agents known in the art for formulation of a protein such as FLINT can also be included in the formulation.

One of ordinary skill in the art will recognize that the methods of the current invention may be achieved by pulmonary administration of FLINT via devices not described herein.

Pharmaceutical Formulations of FLINT

The present invention also relates to a pharmaceutical composition or formulation including FLINT protein suitable for administration by inhalation. The invention also relates to methods for manufacturing formulations including FLINT protein in a form that is suitable for administration by inhalation. For example, a dry powder formulation can be manufactured in several ways, using conventional techniques. Particles in the size range appropriate for maximal deposition in the lower respiratory tract can be made by micronizing, milling, spray drying, or the like. A liquid formulation can be manufactured by dissolving the FLINT

protein in a suitable solvent, such as water, at an appropriate pH, including buffers or other excipients.

Process for Preparing Compositions of the Invention

Another aspect of this invention is a process for preparing a respirable powdered pharmaceutical composition of FLINT. The process comprises preparing a respirable powdered pharmaceutical composition by (a) forming a homogeneous aqueous composition comprising water, a pharmaceutically acceptable excipient and FLINT suitable for treating a disease state by inhalation, (b) removing the water from the aqueous composition to form a solid and (c) transforming the resulting solid into a respirable powdered pharmaceutical composition. In another embodiment, the process further comprises adding a water-soluble, physiologically-acceptable protein, such as human serum albumin (HSA), to the aqueous composition in an amount sufficient to enhance the dispersibility of the resulting respirable powdered FLINT pharmaceutical composition, as described in US Patent 6,187,344 B1, herein incorporated by reference.

In this aspect of the preparation of an aqueous mixture, a solution or stable suspension is formed by dissolving or suspending a suitable excipient, FLINT, and a physiologically acceptable, water-soluble protein such as HSA in water. The order in which the components are added is not of major significance, and while the homogenous mixture may be a solution or suspension, it is preferably a solution.

The proportion of the components in the aqueous mixture is consistent with the proportions that are desired in the

resulting powdered composition. In general, the concentration of the materials is given in the table below:

TABLE I

Suitable Aqueous Compositions

	Range	Preferred range
	mg/100 ml	mg/100 ml
Excipient	15-700	500-700
FLINT	15-700	15-200
Water-soluble Protein	7.5-110	20-40

Another, more specific, aspect of this invention is a method for preparing a spray-dried, dispersible powdered pharmaceutical composition that comprises spray drying a homogeneous aqueous mixture comprising water, a pharmaceutically acceptable excipient, FLINT, and a dispersibility-enhancing amount of a physiologically acceptable, water-soluble protein under conditions sufficient to provide a dispersible powdered pharmaceutical composition having a particle size less than about ten microns.

Therapeutic Use for FLINT

The invention contemplates the administration of therapeutically effective amounts of FLINT via the pulmonary route. What constitutes a therapeutically effective amount of FLINT will depend on the particular disease or condition being treated, and the general medical condition of a patient receiving such treatment. The precise treatment dosage and regimen will depend on a variety of factors which the knowledgeable practitioner will take into account in

arriving at a particular regimen. The amount of FLINT administered may be sufficient to prevent or abate Fas-FasL induced apoptosis, which underlies many of the diseases for which FLINT is useful in treating.

The present invention may be better understood with reference to the following examples. These examples are intended to be representative of specific embodiments of the invention, and are not intended as limiting the scope of the invention.

Generally the amount of active agent present in the composition will vary between about 0.1 % w to about 50% w., preferably from about 0.1% w. to about 5% w. and most preferably from about 0.1% w. to about 2% weight.

The dispersing agent useful in the composition, method and process of this invention and that provides improved dispersibility is a pharmaceutically-acceptable, water-soluble, polypeptide. For purposes of this application, polypeptide is meant to encompass both naturally occurring proteins and artificially constructed polypeptides in which individual amino acid units are linked together through the standard peptide amide bond (the carboxyl group of one and the amino group of another). The dispersing agent is one that can be taken into the lungs of a patient in need thereof but will have no adverse toxicological effects at the levels used. While it is preferable that the dispersing agent be an inactive agent, it is part of this invention to include agents that may have some inherent activity of their own as long as such activity is not antithetical to the utility of the overall composition. The dispersing agent is characterized in having a molecular weight between about 1,000 and about 200,000. An example of an agent having a low molecular weight is a polyalanine

having a molecular weight of about 1000. Other polypeptides in that molecular weight range which are physiologically acceptable but inactive can also be prepared. Molecules that have a molecular weight in the range of about 3000 to 6000 are also useful. If a material is used that has inherent activity of its own, it is used at levels such that the inherent activity does not interfere adversely with the activity of the active agent. Another example representative of the proteins useful in this invention include alpha-lactalbumin, a constituent of milk having a molecular weight of about 14,200. Another example of a representative dispersing agent is trypsinogen, which has a molecular weight of about 24,000. A dispersing agent that is particularly preferred is human serum albumin (HSA), which has a molecular weight of about 68,000. Preferably the molecular weight of the protein dispersing agent is from about 1000 to about 100,000, particularly from about 1,000 to about 70,000 and more particularly about 68,000, i.e., HSA.

The amount of dispersing agent present in the composition of this invention may vary from about 1% w. to about 15% w., preferably from about 3% w. to about 8% w and more preferably from about 3% w to about 5% w.

In addition to the excipient of a carbohydrate, amino acid or mixtures thereof, the active agent and the protein dispersing agent, the composition of this invention may contain other pharmaceutically-acceptable excipients that may be used to stabilize the composition or make it more compatible with the unit dosage form from which it is delivered. Such excipients include, for example, buffers such as citrate, phosphate or acetate.

The composition of this invention will be delivered from a unit dosage receptacle containing an amount that will be sufficient to provide the desired physiological effect upon inhalation by a subject in need thereof. The amount will be dispersed in a chamber that has an internal volume sufficient to capture substantially all of the powder dispersion resulting from the unit dosage receptacle. Usually the volume of the chamber will be from about 50 ml to about 1000 ml, preferably from about 100 ml to about 750 ml. Thus, the unit dosage amount will be from about 2 mg of powder to about 20 mg of powder preferably about 4 mg to about 10 mg of powder per unit dosage. About 5 mg per unit dosage is quite effective. The preferred unit dosage receptacle is a blister pack, generally provided as a series of blister pack strips. The general process for preparing such blister packs or blister pack strips is generally known to one of skill in the art from such publications as Remington's Pharmaceutical Sciences (18th Edition) or other similar publications. The volume of such dosage form receptacle to accommodate the needed amount of powder of this invention will be about 1 ml to about 30 ml, preferably about 2 ml to about 10 ml.

Example 1

Serum Pharmacokinetics of Human FLINT in Beagle Dogs Following Pulmonary Administration of Single Aerosolized Doses

Aerosols of human FLINT generated from solutions of FLINT in sterile water, are administered to anesthetized dogs by the pulmonary route through an endotracheal tube via an ultrasonic nebulizer. Serum concentration of

immunoreactive FLINT are determined by validated radioimmunoassay methods.

Six beagle dogs (3 male and 3 female) are used in the study. Animals are housed either two per cage or individually in stainless steel cages with suspended mesh floors. Initially, all dogs are fed approximately 450 g of Purina Certified Canine Diet 5007 each day. Animals are fasted approximately eight hours before dosing. After recovery from anesthesia, food and water are provided *ad libitum* until 48 hours post-dose. The initial daily feeding regimen is initiated at 48 hours post-dose.

Blood samples are collected at various time points after dosing to determine plasma concentrations of the human FLINT and bioavailability of inhaled material is determined. Dogs are chosen because they are large animals with respiratory tract deposition of particles similar to man.

The effectiveness of pulmonary administration of FLINT is indicated by increased concentrations of immunoreactive FLINT in the serum of treated dogs.

Example 2

Serum Pharmacokinetics of FLINT Analog R218Q in Beagle Dogs Following Pulmonary Administration of Single Aerosolized Doses

Aerosols of human FLINT analog R218Q are generated from a solution of R218Q in sterile water, and are administered to anesthetized dogs by the pulmonary route through an endotracheal tube via an ultrasonic nebulizer. Serum concentration of immunoreactive FLINT R218Q are determined by validated radioimmunoassay methods.

Six beagle dogs (3 male and 3 female) are used in the study. Animals are housed either two per cage or individually in stainless steel cages with suspended mesh floors. Initially, all dogs are fed approximately 450 g of Purina Certified Canine Diet 5007 each day. Animals are fasted approximately eight hours before dosing. After recovery from anesthesia, food and water are provided *ad libitum* until 48 hours post-dose. The initial daily feeding regimen is initiated at 48 hours post-dose.

Blood samples are collected at various time points after dosing to determine plasma concentrations of the FLINT R218Q and bioavailability of inhaled material is determined.

The effectiveness of pulmonary administration of FLINT R218Q is indicated by increased concentrations of immunoreactive FLINT R218Q in the serum of treated dogs.

What is claimed is:

1. A method comprising pulmonary administration of a therapeutically effective amount of FLINT or FLINT analog.

2. A method as in claim 1 wherein said FLINT is delivered to a lower airway of a patient.

3. A method as in claim 2 wherein said FLINT is deposited in the alveoli.

4. A method as in claim 1 wherein said FLINT is inhaled through the mouth of a patient.

5. A method as in claim 1, said FLINT further comprising a pharmaceutically acceptable carrier to form a FLINT composition.

6. A method as in claim 5 wherein said composition is selected from the group consisting of an aqueous solution, an aqueous suspension, a non-aqueous suspension, or a dry powder.

7. A method of claim 6 wherein said composition is administered as an aerosol.

8. A method of claim 6 wherein said composition is a dry powder.

9. A method as in claim 5 wherein said FLINT has a particle size of less than about 10 microns.

10. A method as in claim 5 wherein said FLINT has a particle size of about 1 micron to about 5 microns.

11. A method as in claim 5 wherein said FLINT has a particle size of about 2 microns to about 3 microns.

12. A method as in claim 1 wherein said FLINT is delivered from an inhalation device suitable form pulmonary administration and capable of depositing FLINT in the lungs of a patient.

13. A method of claim 12 wherein said device is selected from the group consisting of a nebulizer, a metered-dose inhaler, a dry-powder inhaler and a sprayer.

14. A method of Claim 1 wherein said analog is R218Q.

SEQUENCE LISTING

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT).

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 February 2002 (07.02.2002)

PCT

(10) International Publication Number
WO 02/09668 A3

(51) International Patent Classification[7]: A61K 9/00

(21) International Application Number: PCT/US01/21105

(22) International Filing Date: 20 July 2001 (20.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/222,476 2 August 2000 (02.08.2000) US

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(74) Agents: **WEBSTER, Thomas, D.** et al.; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
10 May 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/09668 A3

(54) Title: PULMONARY ADMINISTRATION OF FLINT

(57) Abstract: The claimed invention relates to methods and compositions for administering FLINT by inhalation, and a methods for treating a disease by administering FLINT by inhalation.

INTERNATIONAL SEARCH REPORT

Internatl · Application No
PCT/US 01/21105

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 37094 A (ELI LILLY) 29 June 2000 (2000-06-29) claims 1,6,10 page 42, line 5 -page 43, line 5 page 44, line 21 - line 30	1,4-7, 12,13

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance

'E' earlier document but published on or after the international filing date

'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 March 2002	26/03/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ventura Amat, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat' l Application No
PCT/US 01/21105

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0037094 A	29-06-2000	AU 2211100 A	12-07-2000
		AU 3369199 A	18-10-1999
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		CN 1303429 T	11-07-2001
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		PL 343847 A1	10-09-2001
		TR 200002824 T2	21-12-2000
		WO 9950413 A2	07-10-1999
		WO 0037094 A2	29-06-2000